



## Original Article

# The effects of aerobic exercise training on oxidant-antioxidant balance, neurotrophic factor levels, and blood–brain barrier function in obese and non-obese men

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## Abstract

**Purpose:** The purpose of this study was to investigate the effects of obesity and aerobic exercise training on oxidant-antioxidant balance, neurotrophic factor levels, and blood–brain barrier (BBB) function.

**Methods:** Ten non-obese healthy men (body mass index < 25 kg/m<sup>2</sup>) and 10 obese men (body mass index ≥ 25 kg/m<sup>2</sup>) were included in the study. Both groups performed treadmill exercise for 40 min 3 times weekly for 8 weeks at 70% heart rate reserve. Blood samples were collected to examine oxidant-antioxidant balance (reactive oxygen species (ROS) and superoxide dismutase (SOD) activity levels), neurotrophic factors (brain-derived neurotrophic factor (BDNF), nerve growth factor, and glial cell line-derived neurotrophic factor levels), and BBB function (S100β and neuron-specific enolase (NSE) levels) before and after exercise training.

**Results:** The obese group showed significantly greater changes than the non-obese group in serum ROS (−0.46 ± 0.31 mmol/L vs. −0.10 ± 0.17 mmol/L, *p* = 0.005), serum S100β levels (−8.50 ± 5.92 ng/L vs. −0.78 ± 5.45 ng/L, *p* = 0.007), and serum NSE levels (−0.89 ± 0.54 μg/L vs. −0.01 ± 0.74 μg/L, *p* = 0.007) after training. At baseline, the obese group showed significantly higher serum ROS and S100β levels and significantly lower serum SOD activity and BDNF levels than the non-obese group (*p* < 0.05). The obese group showed significantly lower serum ROS, S100β, and NSE levels and significantly higher serum SOD activity and BDNF levels after training compared with the non-obese group (*p* < 0.05).

**Conclusion:** These results suggest that obesity can reduce serum neurotrophic factor levels and can induce BBB dysfunction. On the other hand, aerobic exercise can improve an oxidant-antioxidant imbalance in obese subjects and limit BBB dysfunction.

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**Keywords:** Blood-brain barrier; Exercise training; Neurotrophic factor; Obesity; Oxidative stress; Redox balance

## 1. Introduction

Oxygen is essential to life and physical activity, but it generates toxic reactive oxygen species (ROS) when incompletely reduced.<sup>1,2</sup> These ROS include hydroxyl radical (HO·), hydrogen peroxide, and superoxide radical (O<sub>2</sub><sup>·−</sup>).<sup>2,3</sup> Obesity has been shown to induce oxidative stress and increase ROS by creating an imbalance between pro-oxidants and antioxidants.<sup>4,5</sup>

Obesity is associated with excessive free fatty acid (FFA) levels and hyperglycemia. The excess FFAs are shunted into the citric acid cycle, increasing acetyl-CoA levels and producing nicotinamide adenine dinucleotide dehydrogenase (NADH). A

sudden infusion of FFAs into the body causes an increase in isoprostane levels, a marker of lipid peroxidation and a by-product of cell damage from increased ROS activity.<sup>6</sup> Moreover, increased glucose metabolism by intracellular hyperinsulinemia causes an excessive production of NADH and flavin adenosine dinucleotide, which are used in the electron transport chain to create adenosine triphosphate.<sup>7</sup> The mitochondrial proton gradient increases with excessive NADH, resulting in the creation of superoxide radicals through the transfer of a proton to oxygen. This results in oxidative stress in various organisms.<sup>5,8</sup> Several studies have reported that obese subjects have significantly higher levels of thiobarbituric acid reactive substances, which are markers of plasma oxidative stress.<sup>9,10</sup> They also have significantly decreased glutathione peroxidase and copper-zinc SOD activities,<sup>10,11</sup> worsening oxidative stress.

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The resulting oxidative stress induces DNA denaturation and apoptosis,<sup>12</sup> causing cardiovascular disease, diabetes, cancer, and neurodegenerative diseases.<sup>13,14</sup> In particular, the brain contains significant amounts of unsaturated fatty acid and circulating oxygen but has decreased antioxidant enzyme activity compared with other organs, increasing the risk for the development of neurodegenerative diseases through the apoptosis of vulnerable neurons.<sup>15,16</sup> Moreover, the brain's blood vessels comprise a blood–brain barrier (BBB) composed of tight junctions, pericytes, astrocyte end-feet, and basal lamina. The BBB protects the brain from sudden changes in blood components by selectively blocking toxic substances that threaten normal brain function.<sup>17</sup> However, excessive oxidative stress can damage the BBB<sup>18,19</sup> and result in various neurologic diseases.<sup>20</sup>

Benedict et al.<sup>21</sup> reported that increased peripheral blood levels of neuron-specific enolase (NSE) and S100 $\beta$ , circulating brain-specific proteins, may be indicative of neuronal damage, impaired BBB function, or both. Moreover, obesity regulates neuronal survival, plasticity, and neurotransmitter release and is related to brain-derived neurotrophic factor (BDNF) expression, which can prevent cognitive dysfunction and neurodegenerative diseases.<sup>22</sup> Gardiner et al.<sup>23</sup> suggested that an increase in oxidative stress can be linked with the downregulation of this neurotrophic factor.

On the other hand, regular exercise training is an effective treatment for obesity that reduces oxidative stress caused by obesity or diseases such as metabolic syndrome<sup>24</sup> and induces an increase in neurotrophic factors.<sup>25</sup> However, previous human studies have been limited to the measurement of only BDNF, nerve growth factor (NGF), and glial cell line-derived neurotrophic factor (GDNF).

As noted earlier, regular exercise training can alleviate oxidative stress in obese subjects and can affect neurotrophic factor levels, which promote brain cell growth and support the BBB protecting the brain. However, no previous study has determined the relationship between regular exercise training, oxidative stress, BDNF levels, and BBB damage. Thus, this study aimed to investigate the effects of regular exercise training on the oxidant–antioxidant balance, neurotrophic factor levels, and BBB function in obese subjects.

## 2. Methods

### 2.1. Subjects

Subjects included in this study did not participate in regular exercise and understood the purpose of this study. We included 2 groups: 10 healthy non-obese males with a body mass index (BMI) < 25 kg/m<sup>2</sup> and 10 obese males with a BMI > 25 kg/m<sup>2</sup> based on the World Health Organization/International Association for the Study of Obesity/International Obesity Task Force definition of obesity.<sup>26</sup> The subjects did not take any medication, and no dietary modifications were made during the study. The subjects were informed about data collection and purposes of the study, and all subjects agreed to participate, signing a written informed consent statement. The protocol of this study was approved by the National Research Foundation of Korea

Table 1

Physical characteristics of the subjects included in this study (mean  $\pm$  SD).

Variables	Non-obese (n = 10)	Obese (n = 10)	p*
Age (year)	22.80 $\pm$ 2.35	23.00 $\pm$ 2.36	0.851
Height (cm)	173.90 $\pm$ 3.75	173.20 $\pm$ 4.56	0.712
Weight (kg)	66.63 $\pm$ 3.60	89.25 $\pm$ 10.71	<0.001
BMI (kg/m <sup>2</sup> )	22.00 $\pm$ 1.22	29.74 $\pm$ 3.12	<0.001
Body fat (%)	14.56 $\pm$ 3.29	29.60 $\pm$ 4.37	<0.001
Resting SBP (mmHg)	117.50 $\pm$ 4.60	124.70 $\pm$ 5.08	0.004
Resting DBP (mmHg)	74.50 $\pm$ 6.10	83.10 $\pm$ 7.52	0.012
Resting HR (beats/min)	65.90 $\pm$ 2.73	73.90 $\pm$ 3.93	<0.001
VO <sub>2max</sub> (mL/kg/min)	55.35 $\pm$ 3.10	41.34 $\pm$ 7.36	<0.001

\* Determined using the independent *t* test.

Abbreviations: BMI = body mass index; DBP = diastolic blood pressure; HR = heart rate; SBP = systolic blood pressure; VO<sub>2max</sub> = maximum oxygen uptake.

(NRF-2013S1A5B5A07049580), and the physical characteristics of the subjects are shown in Table 1.

### 2.2. Anthropometric measurements

Anthropometric measurements including height, weight, BMI, percentage of fat, resting blood pressure (BP), resting heart rate (HR), and maximum oxygen uptake (VO<sub>2max</sub>) were obtained. Height was measured using semiautomatic height measurement equipment (HD; STDK, Tokyo, Japan), and weight and body composition were measured using a bioelectrical impedance analysis body composition analyzer (Inbody220; Biospace, Seoul, Korea). Resting BP in the brachial artery was obtained by a nurse with a mercury sphygmomanometer (Trimline; PyMaH, Somerville, NJ, USA) after subjects had relaxed in a comfortably seated position for at least 10 min. The HR was measured with a wireless HR analyzer (Polar A5; Polar, Kempele, Finland). VO<sub>2max</sub> was measured on the treadmill (Q65; Quinton, Seattle, WA, USA) at 1.7 mph and a 10% grade using the Bruce protocol with an increase of 0.8–0.9 mph and 2% grade every 3 min.<sup>27</sup> Breath-by-breath analysis was applied using a gas analyzer (Metamax 3B; Cortex, Leipzig, Germany) and a wireless HR analyzer (Polar A5). Repeat measurements of weight, body composition, resting BP, resting HR, and VO<sub>2max</sub> were conducted after 8 weeks of training to record changes in obesity and cardiovascular parameters.

### 2.3. Exercise training method

Running exercise was performed on a treadmill 3 times weekly for 8 weeks in accordance with a previously described training method<sup>28</sup> and exercise prescription guidelines for obese subjects.<sup>29</sup> Exercise intensity was set at 70%HR reserve using the Karvonen formula, in which the resting HR (HR<sub>rest</sub>) and maximum HR (HR<sub>max</sub>) are measured during the VO<sub>2max</sub> test.<sup>30</sup> Exercise intensity during training was controlled at a  $\pm$ 5% error range of the target HR using the wireless HR analyzer (Polar A5). Exercise duration was 60 min, including 10 min warm-up (stretching) and cool-down periods (stretching) and 40 min of treadmill exercise.

#### 2.4. Blood collection and analysis methods

In all, 10 mL of blood was collected from the antecubital vein with a 22-gauge needle and serum separator tubes before and after 8 weeks of training. The blood separation was performed by centrifugation at 3000 rpm for 15 min, and serum was kept at  $-80^{\circ}\text{C}$  until the analysis of serum oxidant-antioxidant status (ROS and SOD), neurotrophic factors (BDNF, NGF, and GDNF), and BBB function-related factors (S100 $\beta$  and NSE) was performed.

#### 2.5. Blood oxidant–antioxidant marker analysis methods

The analysis of serum ROS was conducted using the OxiSelect *In Vitro* ROS/RNS Assay Kit (#STA-347; Cell Biolabs, San Diego, CA, USA). In this assay 2',7'-dichlorodihydrofluorescein is converted to 2',7'-dichlorodihydrofluorescein diacetate by ROS. Fluorescence was measured at 480 nm and 530 nm using a fluorescence plate reader (LS 55 Luminescence Spectrometer; PerkinElmer, Waltham, MA, USA). The analysis of serum SOD activity was performed using a colorimetric assay with the Superoxide Dismutase Assay Kit (#CM706002; IBL International, Hamburg, Germany) at 450 nm with a microplate reader (GENios; TECAN, Salzburg, Austria).

#### 2.6. Blood neurotrophic factor analysis methods

Serum BDNF, NGF, and GDNF levels were measured using sandwich enzyme-linked immunosorbent assays (ELISAs). For BDNF, we used the Human BDNF ELISA Kit (#DBD00; R&D Systems, Minneapolis, MN, USA); for NGF we used the NGF sandwich ELISA Kit (#CYT304; ChemiKine, Temecula, CA, USA), and for GDNF we used the GDNF Human ELISA Kit (#ab100525; Abcam, Cambridge, MA, USA). Fluorescence was measured at 450 nm with a microplate reader (Emax; Molecular Devices, Sunnyvale, CA, USA).

#### 2.7. BBB function-related marker analysis methods

Serum S100 $\beta$  levels were measured with a S100 $\beta$  (Human) ELISA Kit (#KA0037; Abnova, Taiwan, China), and NSE levels were measured with a Human NSE ELISA Kit (#M-0050; Alpha Diagnostic International, San Antonio, TX, USA).

Fluorescence was measured at 450 nm with a microplate reader (Emax) with ELISA.

#### 2.8. Statistical analysis

The data from this study are expressed as mean  $\pm$  SD using SPSS/PC+ Ver. 21.0 for Windows (IBM, Armonk, NY, USA). Two-way repeated analysis of variance (ANOVA) was conducted to examine the differences in each dependent variable and group before and after exercise training. An independent *t* test was conducted to examine the differences between the obese and non-obese groups prior to training. Statistical significance ( $\alpha$ ) was set at 0.05.

### 3. Results

#### 3.1. Changes in body composition and cardiovascular parameters

Changes in body composition (weight, BMI, and percentage of fat) and cardiovascular parameters (BP, HR, and  $\text{VO}_{2\text{max}}$ ) in the non-obese and obese groups before and after aerobic exercise training are shown in Table 2. The 2-way repeated ANOVA for body composition and  $\text{VO}_{2\text{max}}$  showed an interaction effect in weight ( $F = 16.474$ ,  $p = 0.001$ ), BMI ( $F = 18.384$ ,  $p < 0.001$ ), percentage of fat ( $F = 18.384$ ,  $p < 0.001$ ), and  $\text{VO}_{2\text{max}}$  ( $F = 12.292$ ,  $p = 0.003$ ), which showed significant differences. There were no significant differences in resting systolic blood pressure ( $F = 0.485$ ,  $p = 0.495$ ), diastolic blood pressure ( $F = 1.427$ ,  $p = 0.248$ ), and HR ( $F = 1.458$ ,  $p = 0.243$ ). The *post hoc* test results revealed that the non-obese group did not show significant differences in these parameters before and after training, but the obese group showed significantly lower weight, BMI, and percentage of fat (all  $p < 0.05$ ) and a significantly higher  $\text{VO}_{2\text{max}}$  ( $p < 0.05$ ) after training. Moreover, the obese group showed a significantly higher weight, BMI, and percentage of fat and a significantly lower  $\text{VO}_{2\text{max}}$  before and after training compared with the non-obese group (all  $p < 0.05$ ).

#### 3.2. Changes in blood oxidant–antioxidant balance

Changes in the oxidant–antioxidant balance in the nonobese and obese groups before and after aerobic exercise training are shown in Table 3. The 2-way repeated ANOVA revealed

Table 2  
Changes in body composition and cardiovascular assessments before and after training (mean  $\pm$  SD).

Variables	Non-obese ( $n = 10$ )				Obese ( $n = 10$ )				Time $\times$ group interaction	
	Baseline	CV	After training	CV	Baseline	CV	After training	CV	<i>F</i>	<i>p</i>
Weight (kg)	66.63 $\pm$ 3.60	0.05	66.43 $\pm$ 3.70	0.06	89.25 $\pm$ 10.71*	0.12	84.36 $\pm$ 12.11*. <sup>#</sup>	0.14	16.474	0.001
BMI (kg/m <sup>2</sup> )	22.00 $\pm$ 1.22	0.06	21.87 $\pm$ 1.24	0.06	29.74 $\pm$ 3.12*	0.11	27.95 $\pm$ 3.30*. <sup>#</sup>	0.12	18.384	<0.001
Body fat (%)	14.56 $\pm$ 3.29	0.23	13.98 $\pm$ 3.12	0.22	29.60 $\pm$ 4.37*	0.15	26.59 $\pm$ 4.97*. <sup>#</sup>	0.19	18.384	<0.001
Resting SBP (mmHg)	117.50 $\pm$ 4.60	0.04	117.40 $\pm$ 4.40	0.04	124.70 $\pm$ 5.08	0.04	123.10 $\pm$ 5.40	0.04	0.485	0.495
Resting DBP (mmHg)	74.50 $\pm$ 6.10	0.08	73.10 $\pm$ 4.56	0.06	83.10 $\pm$ 7.52	0.09	79.10 $\pm$ 6.24	0.08	1.427	0.248
Resting HR (beats/min)	65.90 $\pm$ 2.73	0.04	65.10 $\pm$ 3.00	0.05	73.90 $\pm$ 3.93	0.05	71.50 $\pm$ 3.57	0.05	1.458	0.243
$\text{VO}_{2\text{max}}$ (mL/kg/min)	55.35 $\pm$ 3.10	0.06	55.98 $\pm$ 3.48	0.06	41.34 $\pm$ 7.36*	0.18	46.98 $\pm$ 8.14*. <sup>#</sup>	0.17	12.292	0.003

\*  $p < 0.05$ , compared with corresponding values in non-obese group; <sup>#</sup>  $p < 0.05$ , compared with baseline values within obese group.

Abbreviations: BMI = body mass index; CV = coefficient of variation; DBP = diastolic blood pressure; HR = heart rate; SBP = systolic blood pressure;  $\text{VO}_{2\text{max}}$  = maximum oxygen uptake.

Table 3  
Changes in blood oxidant–antioxidant status before and after training (mean  $\pm$  SD).

Variables	Non-obese ( $n = 10$ )				Obese ( $n = 10$ )				Time $\times$ group interaction	
	Baseline	CV	After training	CV	Baseline	CV	After training	CV	$F$	$p$
ROS (mmol/L)	1.52 $\pm$ 0.34	0.23	1.41 $\pm$ 0.23	0.16	1.90 $\pm$ 0.34*	0.18	1.43 $\pm$ 0.26 <sup>#</sup>	0.18	10.209	0.005
SOD (U/mL)	2.76 $\pm$ 0.53	0.19	2.83 $\pm$ 0.50	0.18	2.10 $\pm$ 0.72 <sup>#</sup>	0.34	2.76 $\pm$ 0.58 <sup>#</sup>	0.21	9.502	0.006

\*  $p < 0.05$ , compared with baseline values in non-obese group; <sup>#</sup>  $p < 0.05$ , compared with baseline values within obese group.

Abbreviations: CV = coefficient of variation; ROS = reactive oxygen species; SOD = superoxide dismutase.

significant differences in blood ROS levels and SOD activity between the 2 groups before and after training ( $F = 10.209$ ,  $p = 0.005$ ;  $F = 9.502$ ,  $p = 0.006$ , respectively). The *post hoc* test results showed that the obese group had significantly higher ROS levels and lower SOD activity at baseline compared with the non-obese group (both  $p < 0.05$ ). In addition, the non-obese group showed no significant differences in these values before and after training, whereas the obese group showed significantly decreased ROS levels and increased SOD activity after training (both  $p < 0.05$ ).

### 3.3. Changes in blood neurotrophic factor levels

Changes in blood neurotrophic factor levels in the non-obese and obese groups before and after aerobic exercise training are shown in Table 4. The 2-way repeated ANOVA showed interaction effects for blood BDNF before and after training, indicating a significant difference ( $F = 7.665$ ,  $p = 0.013$ ). There were no significant differences in blood NGF and GDNF levels ( $F = 1.802$ ,  $p = 0.196$ ;  $F = 1.854$ ,  $p = 0.190$ , respectively). According to the *post hoc* test results, the obese group showed a significantly lower BDNF level at baseline compared with the non-obese group ( $p < 0.05$ ). In addition, the non-obese group showed no significant difference in blood neurotrophic factor levels before and after training, whereas the obese group showed a significantly higher BDNF level after training ( $p < 0.05$ ).

### 3.4. Changes in serum BBB function markers

Changes in serum BBB function markers in the non-obese and obese groups before and after aerobic exercise training are shown in Table 5. The 2-way ANOVA showed interaction effects for blood S100 $\beta$  and NSE levels before and after training, indicating significant differences ( $F = 9.202$ ,  $p = 0.007$ ;  $F = 9.271$ ,  $p = 0.007$ , respectively). According to the *post hoc* test results, the obese group showed significantly higher S100 $\beta$  levels at baseline compared with the non-obese group ( $p < 0.05$ ). Furthermore, the non-obese group showed no significant differences before or after training, whereas the obese group showed significantly lower S100 $\beta$  levels and NSE levels after training (both  $p < 0.05$ ).

## 4. Discussion

Regular exercise, along with dietary control, is effective in the prevention of obesity.<sup>31</sup> In our study, body composition parameters including weight, BMI, and percentage of fat were significantly reduced in the obese group after training, indicating a positive effect of training on obesity reduction. In addition, the obese group showed a significantly increased  $VO_{2max}$ , whereas the non-obese group showed no significant change in  $VO_{2max}$  with training. We believe this occurred because the  $VO_{2max}$  of the non-obese group was in the upper 10th percentile before training ( $VO_{2max} > 51.4$  mL/kg/min),<sup>32</sup> and therefore a

Table 4  
Changes in blood neurotrophic factor levels before and after training (mean  $\pm$  SD).

Variables	Non-obese ( $n = 10$ )				Obese ( $n = 10$ )				Time $\times$ group interaction	
	Baseline	CV	After training	CV	Baseline	CV	After training	CV	$F$	$p$
BDNF (pg/mL)	29,777.40 $\pm$ 3243.85	0.11	30,121.62 $\pm$ 2974.32	0.10	24,501.20 $\pm$ 3951.45*	0.16	29,508.34 $\pm$ 4817.02 <sup>#</sup>	0.16	7.665	0.013
NGF (pg/mL)	373.60 $\pm$ 39.28	0.11	398.94 $\pm$ 22.96	0.06	366.99 $\pm$ 35.66	0.10	373.81 $\pm$ 14.98	0.04	1.802	0.196
GDNF (pg/mL)	634.96 $\pm$ 111.78	0.18	650.17 $\pm$ 89.54	0.14	586.05 $\pm$ 64.44	0.11	638.42 $\pm$ 76.41	0.12	1.854	0.190

\*  $p < 0.05$ , compared with the baseline value in non-obese group; <sup>#</sup>  $p < 0.05$ , compared with the baseline value within obese group.

Abbreviations: BDNF = brain-derived neurotrophic factor; CV = coefficient of variation; GDNF = glial cell line-derived neurotrophic factor; NGF = nerve growth factor.

Table 5  
Changes in blood blood-brain barrier function markers before and after training (mean  $\pm$  SD).

Variables	Non-obese ( $n = 10$ )				Obese ( $n = 10$ )				Time $\times$ group interaction	
	Baseline	CV	After training	CV	Baseline	CV	After training	CV	$F$	$p$
S100 $\beta$ (ng/L)	49.03 $\pm$ 11.83	0.24	48.25 $\pm$ 9.69	0.20	63.20 $\pm$ 14.56*	0.23	54.70 $\pm$ 10.47 <sup>#</sup>	0.19	9.202	0.007
NSE ( $\mu$ g/L)	5.99 $\pm$ 1.13	0.19	5.98 $\pm$ 1.09	0.18	6.87 $\pm$ 0.90	0.13	5.98 $\pm$ 0.87 <sup>#</sup>	0.14	9.271	0.007

\*  $p < 0.05$ , compared with the baseline value in non-obese group; <sup>#</sup>  $p < 0.05$ , compared with baseline values within obese group.

Abbreviations: CV = coefficient of variation; NSE = neuronspecific enolase.



greater exercise effort was required to promote cardiorespiratory fitness in the non-obese group.

Oxidative stress occurs when pro-oxidants are predominant compared with antioxidants,<sup>33</sup> and it has been reported that obesity is associated with chronically increased oxidative stress.<sup>24</sup> In this study, the obese group had a significantly higher ROS level and significantly lower SOD activity at baseline compared with the non-obese group. This result supports previous studies reporting that obese subjects show a higher pro-oxidant/antioxidant ratio compared with that of normal-weight subjects<sup>34</sup> and implies that obesity can increase oxidative stress. On the other hand, ROS levels decreased and SOD activity levels increased significantly in the obese group after training. It is assumed that exercise training improved the antioxidant balance in the obese group as well as significantly decreasing weight and BMI. Vincent and Taylor<sup>5</sup> reported that obesity induces an imbalance in pro-oxidant–antioxidant imbalance by depleting enzymatic antioxidants such as SOD, but that exercise training increases antioxidant status, and weight loss ameliorates increased oxidative stress in obese subjects. In addition, according to a large epidemiologic study, obesity is highly associated with BMI and oxidative stress, supporting the results of this study.<sup>35</sup> Moreover, high blood glucose levels may be associated with obesity and increased oxidative stress, but glucose levels were not assessed in this study. This is a limitation of our study, and future studies should examine the association between glucose levels, exercise training, and oxidative stress.

Recent studies have reported that obesity can increase the body's oxidative stress. High oxidative stress reduces neurotrophic factor levels, has a negative impact on brain function, and is related to the occurrence of neurodegenerative diseases.<sup>36,37</sup> In this study, we analyzed serum BDNF, NGF, and GDNF levels to examine the effects of obesity and exercise training on neurotrophic factor levels. The obese group showed significantly lower serum BDNF levels at baseline compared with those of the non-obese group, but BDNF levels in the obese group increased significantly after exercise training. This result supports those of previous studies, which reported that obese and overweight subjects showed significantly lower serum BDNF levels compared with normal-weight subjects<sup>38,39</sup> and that aerobic exercise training significantly increased serum BDNF levels in obese subjects.<sup>39,40</sup> The changes in serum ROS levels and SOD activity in this study indicate that the reduced oxidative stress and improved antioxidant ability with exercise training can significantly affect BDNF levels. Increased oxidative stress levels can downregulate neurotrophic factors,<sup>23</sup> and BDNF has shown a negative correlation with oxidative stress and a positive correlation with antioxidant activity.<sup>41,42</sup> Moreover, recent studies have shown improved antioxidant activity with regular exercise and that the use of various antioxidants can promote the expression of neurotrophic factors,<sup>43–45</sup> supporting the results of this study. On the other hand, there was a significant difference in BDNF levels between the obese and non-obese groups at baseline. The obese group showed significant increases in BDNF after training, but no increase was observed in NGF and GDNF levels. These results support those

of a previous study reporting that exercise training significantly increased hippocampal BDNF mRNA expression but not NGF and GDNF mRNA expression in rats.<sup>46</sup> NGF, GDNF, and BDNF levels play an important role in the survival, maintenance, and regeneration of a specific neuronal population.<sup>47</sup> BDNF also plays an important role in the central and peripheral molecular processes of energy metabolism and homeostasis.<sup>45,48</sup> Additional studies are needed to determine the effects of calorie restriction on these neurotrophic factors.

The BBB is a multicellular vascular structure that separates the central nervous system from the peripheral blood circulation. The BBB actively regulates influx and efflux at the blood–brain interface.<sup>49</sup> A disruption of the BBB may play a role in the etiology of various cerebrovascular and nervous diseases such as ischemic stroke, epilepsy, amyotrophic lateral sclerosis, and neuromyelitis optica.<sup>49</sup> Thus, the maintenance of BBB function is important for long-term brain health. In this study, we measured serum S100 $\beta$  and NSE levels to examine the effects of obesity and exercise training on BBB function. The obese group showed significantly higher serum S100 $\beta$  levels at baseline ( $63.20 \pm 14.56$  ng/L) compared with those of the non-obese group ( $49.03 \pm 11.83$  ng/L). This correlation between obesity and serum S100 $\beta$  levels was similar to that reported by Steiner et al.,<sup>50</sup> who showed that overweight subjects with a BMI of 25–29.9 kg/m<sup>2</sup> showed significantly higher serum S100 $\beta$  levels (about 60 ng/L) compared with control subjects (about 50 ng/L) with a BMI of 25 kg/m<sup>2</sup>. Also, S100 $\beta$  and NSE levels were significantly reduced in the obese group after exercise training. These results show that obesity exacerbates BBB dysfunction by increasing oxidative stress, but exercise training reduces ROS levels and increases SOD activity. Microglia activation, the upregulation of proinflammatory cytokines, and an increase in oxidative stress largely account for the obesity-induced BBB disruption.<sup>18</sup> However, antioxidants such as catalase and SOD have been shown to attenuate the BBB hyperpermeability resulting from hyperglycemia.<sup>51</sup> Schulpis et al.<sup>52</sup> reported a negative correlation between serum S100 $\beta$  levels and total antioxidant status ( $r = -0.64$ ). Moreover, Al-Jarrah and Jamous<sup>53</sup> previously reported that treadmill exercise training decreased S100 $\beta$  and NSE expression in a Parkinson disease mouse model, supporting the results of this study. In addition, even though serum S100 $\beta$  and NSE levels are blood biomarkers for BBB disruption and a BBB permeability increase,<sup>21,54</sup> future studies analyzing circulating tight junction proteins and matrix metalloproteinases may more specifically reflect BBB function because S100 $\beta$  is expressed in various peripheral tissues, including skeletal muscle, and NSE reflects brain damage.<sup>55,56</sup>

## 5. Conclusion

In conclusion, obesity can reduce serum neurotrophic factor levels and induce BBB dysfunction. Increased oxidative stress caused by obesity is largely responsible for this phenomenon. On the other hand, regular aerobic exercise can improve the oxidant–antioxidant imbalance resulting from obesity, increase neurotrophic factor levels, and limit BBB dysfunction. However, future studies should investigate calorie restriction

because neurotrophic factors, such as BDNF, are also involved in energy metabolism,<sup>45,48</sup> and serum S100 $\beta$  levels show a high correlation with changes in weight.<sup>50</sup>

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### Authors' contributions

HTR participated in study design, subject recruitment, data collection, data processing, and data analysis and drafted the manuscript; WYS conceived of the study, participated in its design and coordination, and helped draft the manuscript. Both authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

### Competing interests

Neither of the authors declares competing financial interests.

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